[CANCER RESEARCH 59, 590–596, February 1, 1999]

Quantitation of Urinary Metabolites of a Tobacco-specific Lung Carcinogen after Smoking Cessation[1]

Stephen S. Hecht,[2] Steven G. Carmella, Menglan Chen, J. F. Dor Koch, Andrew T. Miller, Sharon E. Murphy, Joni A. Jensen, Cheryl L. Zimmerman, and Dorothy K. Hatsukami

University of Minnesota Cancer Center [S. S. H., S. G. C., M. C., J. F. D. K., A. T. M., S. E. M.], College of Pharmacy [C. L. Z.], and Department of Psychiatry, University of Minnesota [J. A. J., D. K. H.], Minneapolis, Minnesota 55455

ABSTRACT

We quantified urinary levels of two metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in people who had stopped smoking: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its *O*-glucuronide, 4-[(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-*O*-D-glucopyranosiduronic acid (NNAL-Gluc). Twenty-seven people completed the study. Thirteen used the nicotine patch starting at the quit date, whereas the others used no patch. Two 24-h urine samples were collected on 2 consecutive days before smoking cessation; blood was also obtained. Beginning at their quit date, subjects provided 24-h urine samples on days 7, 21, 42, 70, 98, and 126, and some subjects also provided samples at later times. The urine was analyzed for NNAL, NNAL-Gluc, nicotine plus nicotine-*N*-glucuronide, and cotinine plus cotinine-*N*-glucuronide. Some blood samples were also analyzed for NNAL. The decline of urinary NNAL and NNAL-Gluc after smoking cessation was much slower than expected. This was clearly demonstrated by comparisons with cotinine and nicotine levels in urine. One week after smoking cessation, 34.5% of baseline NNAL plus NNAL-Gluc was detected in urine, whereas the corresponding values for cotinine and nicotine were 1.1 and 0.5%, respectively. Even 6 weeks after cessation, 7.6% of the original levels of NNAL plus NNAL-Gluc remained. In some subjects, NNAL plus NNAL-Gluc were detected 281 days after cessation. The distribution half-life for NNAL and NNAL-Gluc was 3–4 days, whereas the elimination half-life was 40–45 days. Total body clearance of NNAL was estimated to be 61.4 ± 35.4 ml/min, and volume of distribution in the β-phase was estimated to be 3500 ± 2100 liters, indicating substantial distribution into the tissues. Parallel studies in rats treated chronically or acutely with NNK in the drinking water support the conclusion that NNAL has a large volume of distribution. There was no effect of the nicotine patch on levels of NNAL plus NNAL-Gluc, indicating that NNK is not formed endogenously from nicotine. The results of this study demonstrate that NNAL and NNAL-Gluc are slowly cleared from the body after smoking cessation, indicating the presence of a high-affinity compartment where NNK, NNAL, and/or NNAL-Gluc are retained or sequestered and slowly released.

INTRODUCTION

In this study, we quantified two metabolites, NNAL[3] and NNAL-Gluc, of the tobacco-specific lung carcinogen NNK in the urine of people who had stopped smoking for 4 months. NNK is believed to play a significant role as a cause of lung cancer in smokers (1–3). NNK is a potent pulmonary carcinogen in rodents, inducing adenocarcinoma of the lung, independent of the route of administration (1). The lowest total doses of NNK that can produce lung tumors in rats are similar to the lifetime total dose of NNK in a smoker (1). Biochemical studies indicate that the same routes of metabolic activation and detoxification of NNK exist in rodents and humans, although there are quantitative differences (1). Major pathways of NNK metabolism are summarized in Fig. 1 (1). In rodents and humans, NNK is rapidly metabolized to its carbonyl reduction product NNAL, which is also a pulmonary carcinogen in rats and mice. NNAL is *O*-glucuronidated to give NNAL-Gluc, which is excreted in urine. Urinary NNAL and NNAL-Gluc are excellent biomarkers for NNK uptake in smokers and in people exposed to environmental tobacco smoke (1). NNK itself is not detected in urine due to its extensive metabolism. Both NNAL and NNK undergo metabolic hydroxylation at the carbons adjacent to the nitrosamino nitrogen. These cytochrome P450-catalyzed α-hydroxylation reactions generate intermediates that react with DNA, causing permanent mutations associated with tumor induction (1).

Little is known about the excretion of carcinogens and other tobacco smoke constituents after smoking cessation. Two studies examined the decrease of 4-aminobiphenyl hemoglobin adducts after withdrawal from smoking; the decline was faster than predicted, based on the lifetime of the red cell (4, 5). Polycyclic aromatic hydrocarbon-DNA adducts in leukocytes, as determined by immunoassay, also decreased after smoking cessation (5). Other indicators of DNA damage, such as glycophorin A mutations or BPDE-DNA adduct antibodies, were unaffected by cessation (5, 6). Among urinary constituents related to smoking, only 8-oxodeoxyguanosine and cotinine appear to have been studied. 8-Oxodeoxyguanosine, a product of oxidative damage by cigarette smoke, decreased by 21% 4 weeks after cessation (7). Urinary cotinine, a major metabolite of nicotine, is reported to have a half-life of 16.5 h after cessation (8). NNK and NNAL are structurally related to nicotine and cotinine. On this basis alone, one would expect fairly rapid elimination of NNK metabolites after smoking cessation. However, our preliminary data suggested that this was not the case. Therefore, we investigated the persistence of NNAL and NNAL-Gluc in people who had quit smoking. As the results will show, they were far more persistent than either nicotine or cotinine, suggesting that NNK or NNAL was slowly eliminated from the body.

MATERIALS AND METHODS

Study Design. The study was approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee. The study design is summarized in Fig. 2. Smokers were recruited through newspaper advertisements and local press releases. Subjects were initially screened over the telephone. They must have smoked at least 15 cigarettes per day for the past year. Potential subjects were scheduled for a screening visit at the University of Minnesota Tobacco Research Programs clinic. At the screening visit, they filled out a questionnaire regarding their smoking history and completed a medical history form. Smoking status was confirmed by salivary cotinine and expired CO. Subjects were excluded if they had any current medical condition, used prescribed medications, or had a psychiatric diagnosis. Qualified subjects smoked normally for 7 days prior to their quit date. Two 24-h urine samples were collected on 2 consecutive days during this period and analyzed for nicotine, cotinine, NNAL, and NNAL-Gluc. Blood samples were also obtained. Subjects reported to the study clinic on the evening before their quit date. Data were collected on vital signs,

Received 8/19/98; accepted 12/2/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported by National Cancer Institute Grant CA-44377.

[2] To whom requests for reprints should be addressed, at University of Minnesota Cancer Center, Box 806 Mayo, 420 Delaware Street SE, Minneapolis, MN 55455.

[3] The abbreviations used are: NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, 4-[(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-*O*-D-glucopyranosiduronic acid; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; GC-TEA, gas chromatography-nitrosamine-selective detection; TMS, trimethylsilyl ether.

PM3001192660

Source: https://www.industrydocuments.ucsf.edu/docs/rnwk0001